



2066983

Accession No. 405399-05

## DATA EVALUATION RECORD

1. **CHEMICAL:** Verbenone. Shaughnessey Number: 128986.
2. **TEST MATERIAL:** Verbenone; 100% active ingredient; a clear liquid.
3. **STUDY TYPE:** Freshwater Invertebrate Acute Toxicity Test.  
Species Tested: Daphnia magna.
4. **CITATION:** Surprenant, D.C. 1988. Static Acute Toxicity of Verbenone to Daphnids (Daphnia magna). Submitted by Phero Tech, Inc., Vancouver, B.C., Canada. Study performed by Springborn Life Sciences, Inc., Wareham, MA. Laboratory Report No. 88-2-2627. EPA Accession No. 405399-05.
5. **REVIEWED BY:**  
  
Michael L. Whitten, M.S.  
Wildlife Toxicologist  
KBN Engineering and  
Applied Sciences, Inc.  
  
Signature: *Michael L. Whitten*  
Date: 8-10-89
6. **APPROVED BY:**  
  
Isabel C. Johnson, M.S.  
Principal Scientist  
KBN Engineering and  
Applied Sciences, Inc.  
  
Signature: *Isabel C. Johnson*  
Date: August 11, 1989  
  
Henry T. Craven, M.S.  
Supervisor, EEB/HED  
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Signature:  
Date:
7. **CONCLUSIONS:** The study is scientifically sound but does not fulfill the requirements for a 48-hour static acute toxicity study. Based upon nominal concentrations, the 48-hour EC50 of Verbenone to daphnids was 200 mg/L. This value classifies Verbenone as practically non-toxic to daphnids. The NOEC was determined to be 130 mg/L. Since the test chemical was partially insoluble, the actual concentrations to which the daphnids were exposed is unknown.
8. **RECOMMENDATIONS:** N/A

9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A

11. **MATERIALS AND METHODS:**

A. **Test Animals:** *Daphnia magna* used in this test were obtained from laboratory stocks cultured at the testing facility. The daphnids were cultured in a temperature controlled area at  $20 \pm 1^\circ\text{C}$ . During the holding period, the daphnids were fed a suspension of algae (*Ankistrodesmus falcatus*) and yeast suspension once daily. Only daphnids  $\leq 24$  hours old were selected for testing.

B. **Test System:** The test was conducted in 250 mL glass beakers. The test solution depth was 6.2 cm with a surface area of  $33 \text{ cm}^2$ . Test solutions were prepared by adding the appropriate quantity of Verbenone directly to test vessels containing 200 mL of hard reconstituted water. Each solution was then mixed for approximately 30 seconds by stirring with a glass rod. Four control beakers were established and maintained under the same conditions as the test beakers but containing no Verbenone.

The dilution water was prepared by fortifying well water and filtering it through an Amberlite XAD-7 resin column to remove any potential organic contaminants. The dilution water had a total hardness of 180 mg/L as  $\text{CaCO}_3$ , a total alkalinity of 120 mg/L as  $\text{CaCO}_3$ , a pH of 8.2, and a specific conductivity of 420  $\mu\text{mhos/cm}$ .

The ambient air temperature in the culture area was controlled in order to maintain test solution temperatures at  $21 \pm 1^\circ\text{C}$ . A photoperiod of 16 hours of light and 8 hours of darkness was provided each day.

C. **Dosage:** 48-hour acute static test.

D. **Design:** A control and five nominal concentrations of 130, 220, 360, 600, and 1000 mg/L were tested. Five daphnids were impartially distributed into each of four replicate test and control chambers (20 per concentration) within 20 minutes after the test solutions had been prepared. Daphnids were not fed during the test.

Biological observations of all concentrations were conducted at 0, 24, and 48 hours. The number of immobilized daphnids was recorded at 24 and 48 hours. Water quality parameters (temperature, dissolved oxygen and pH) were measured in one vessel of each treatment level and the control at 0, 24, and 48 hours.

E. **Statistics:** The EC50 and 95% confidence limits were determined for 24- and 48-hour exposure periods using the computer program developed by Stephan (Peltier, 1985). Immobilization of the daphnids was the effect under investigation.

12. **REPORTED RESULTS:** The cumulative percentages of immobilized daphnids are presented in Table 1 (attached). At test initiation a precipitate was observed on the surface of the 130, 220, 360, and 600 mg/L test solutions and a film was present on the surface of the 1000 mg/L solutions. Throughout the remainder of the exposure period a film of undissolved test material was present on the surface of all solutions containing Verbenone.

Using probit analysis, the 24-hour EC50 was estimated to be 340 mg/L with a 95% confidence interval of 300 to 390 mg/L. The 48-hour EC50 was estimated by nonlinear interpolation to be 200 mg/L with a 95% confidence interval calculated by binomial probability of 130 to 360 mg/L. Based on the results of this study, Verbenone is considered practically non-toxic to Daphnia magna.

The dissolved oxygen concentrations, pH, and temperature measured during the test are shown in Table 3 (attached). The dissolved oxygen concentration remained at or above 92% of saturation throughout the test. The pH ranged from 8.0 to 8.2 during the test.

Daphnids on the bottom of the test vessels were observed in the 220 and 360 mg/L test concentrations after 24 hours. The NOEC was 130 mg/L.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**

The author presented no conclusions other than various remarks reported in Section 12, above.

Quality Assurance and Good Laboratory Practice Regulation Statements were included in the report. The director stated that the study followed "...all pertinent EPA Good Laboratory Practice regulations except in the case of

characterization and verification of the test substance identity."

14. **REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:**

- A. **Test Procedure:** The test procedures were in accordance with recommended protocols with the following exceptions:

The hardness of the dilution water was 180 mg/L as  $\text{CaCO}_3$ ; the SEP recommends 40 to 48 mg/L.

The SEP states that if test material solubility problems are encountered (i.e., a precipitate is present), chemical analyses are required to verify test exposure concentrations. Test concentrations were not measured in this study, although precipitates and surface films were reported.

- B. **Statistical Analysis:** Using EPA's Toxanal computer program, the 48-hour EC50 was estimated by the binomial test to be 200 mg/L with a 95% confidence interval of 130 to 360 mg/L (attached). This EC50 and confidence interval are the same as reported by the author.

- C. **Discussion/Results:** At test initiation a precipitate was observed on the surface of all treatment solutions except at 1000 mg/L, and a film was present on the surface of the 1000 mg/L solutions. Throughout the remainder of the exposure period a film of undissolved test material was observed on the surface of all solutions containing Verbenone. The partial insolubility of the test chemical indicates that the actual concentrations of toxicant were less than the nominal values.

The 48-hour EC50 of 200 mg/L (based upon nominal concentrations) classifies Verbenone as practically non-toxic to Daphnia magna. The study appears to be scientifically valid, but since the test chemical was partially insoluble, the actual concentrations to which the daphnids were exposed is unknown.

D. Adequacy of the Study:

- (1) **Classification:** Supplemental
- (2) **Rationale:** Problems with solubility preclude a proper determination of toxicant concentrations.
- (3) **Repairability:** Yes, if the registrant can show that the concentration of Verbenone was not seriously less than the nominal concentrations. An LC50 of greater than 100 mg/L would still classify Verbenone as practically non-toxic to daphnids.

15. COMPLETION OF ONE-LINER: Yes, 8-2-89.

Table 1. Concentrations tested and corresponding cumulative percent of immobilized organisms and observations made during the 48-hour static exposure of daphnids (Daphnia magna) to Verbenone.

Nominal Concentration (mg/L)	Cumulative Number of Immobilized Organisms (%)									
	24-hour					48-hour				
	A	B	C	D	Mean	A	B	C	D	Mean
Control	0	0	0	0	0	0	0	0	0	0
130	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>	0	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>	0
220	0 <sup>a</sup>	0 <sup>a</sup>	20 <sup>ac</sup>	20 <sup>ad</sup>	10	20 <sup>a</sup>	40 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	65
360	0 <sup>ab</sup>	100 <sup>a</sup>	0 <sup>ab</sup>	100 <sup>a</sup>	50	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100
600	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100
1000	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100 <sup>a</sup>	100

<sup>a</sup>A film was present on the surface of the test solution.

<sup>b</sup>All of the surviving daphnids were on the bottom of the test solution.

<sup>c</sup>Two of the surviving daphnids were on the bottom of the test solution.

<sup>d</sup>Several of the surviving daphnids were on the bottom of the test solution.

Table 3. pH, dissolved oxygen concentration, and temperature measurements made during the 48-hour static exposure of daphnids (Daphnia magna) to Verbenone.

Nominal Concentration (mg/L)	0-hour	24-hour	48-hour
<u>pH</u>			
Control	8.0	8.2	8.0
130	8.1	8.2	8.0
220	8.1	8.2	8.1
360	8.1	8.2	8.1
600	8.1	8.2	--- <sup>a</sup>
1000	8.1	8.2	---
<u>Dissolved Oxygen, mg/L</u> (% saturation)			
Control	9.0(99)	8.7(95)	5.8(64)
130	9.0(99)	8.6(94)	5.4(59)
220	9.0(99)	8.6(94)	5.8(64)
360	9.0(99)	8.4(92)	5.8(64)
600	9.0(99)	8.5(93)	---
1000	9.0(99)	8.5(93)	---
<u>Temperature (°C)<sup>b</sup></u>			
Control	20	20	20

<sup>a</sup>Measurement not required at stated time interval

<sup>b</sup>Temperature measurements of exposure solutions were identical to the control values at each 24 hour observation interval.

WHITTEN VERBENONE DAPHNIA MAGNA 08-02-89

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINDMIAL PROB. (PERCENT)
1000	20	20	100	9.536742E-05
600	20	20	100	9.536742E-05
360	20	20	100	9.536742E-05
220	20	13	65	13.1588
130	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 130 AND 360 CAN BE  
USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT  
CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL  
ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 200.4596

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE  
PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE  
NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

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Assay No. 128986

Chemical Name Verbenone Chemical Class \_\_\_\_\_ Page 1 of 1

Study/Species/Lab/  
Accession X a.i.

Reviewer/  
Date \_\_\_\_\_  
Vall.  
St. \_\_\_\_\_

Results

14-Day Single Dose Oral LD<sub>50</sub>

LD<sub>50</sub> = mg/kg ( 95% C.L. ) Contr. Mort.(X) = \_\_\_\_\_

Species \_\_\_\_\_

Slope = \_\_\_\_\_ # Animals/Level = \_\_\_\_\_ Age(Days) = \_\_\_\_\_  
Sex = \_\_\_\_\_

Lab \_\_\_\_\_

14-Day Dose Level mg/kg/(X Mortality)  
( ) , ( ) , ( ) , ( ) , ( ) , ( ) , ( ) , ( )

Acc. \_\_\_\_\_

Comments: \_\_\_\_\_

14-Day Single Dose Oral LD<sub>50</sub>

LD<sub>50</sub> = mg/kg ( 95% C.L. ) Contr. Mort.(X) = \_\_\_\_\_

Species \_\_\_\_\_

Slope = \_\_\_\_\_ # Animals/Level = \_\_\_\_\_ Age(Days) = \_\_\_\_\_  
Sex = \_\_\_\_\_

Lab \_\_\_\_\_

14-Day Dose Level mg/kg/(X Mortality)  
( ) , ( ) , ( ) , ( ) , ( ) , ( ) , ( ) , ( )

Acc. \_\_\_\_\_

Comments: \_\_\_\_\_

8-Day Dietary LC<sub>50</sub>

LC<sub>50</sub> = ppm ( 95% C.L. ) Contr. Mort.(X) = \_\_\_\_\_

Species \_\_\_\_\_

Slope = \_\_\_\_\_ # Animals/Level = \_\_\_\_\_ Age(Days) = \_\_\_\_\_  
Sex = \_\_\_\_\_

Lab \_\_\_\_\_

8-Day Dose Level ppm/(X Mortality)  
( ) , ( ) , ( ) , ( ) , ( ) , ( ) , ( ) , ( )

Acc. \_\_\_\_\_

Comments: \_\_\_\_\_

8-Day Dietary LC<sub>50</sub>

LC<sub>50</sub> = ppm ( 95% C.L. ) Contr. Mort.(X) = \_\_\_\_\_

Species \_\_\_\_\_

Slope = \_\_\_\_\_ # Animals/Level = \_\_\_\_\_ Age(Days) = \_\_\_\_\_  
Sex = \_\_\_\_\_

Lab \_\_\_\_\_

8-Day Dose Level ppm/(X Mortality)  
( ) , ( ) , ( ) , ( ) , ( ) , ( ) , ( ) , ( )

Acc. \_\_\_\_\_

Comments: \_\_\_\_\_

48-Hour LC<sub>50</sub>

LC<sub>50</sub> = 200 \* PP.M ( 95% C.L. ) Contr. Mort.(X) = D  
130-360 Sol. Contr. Mort.(X) = N/A

Species Daphnia magna  
100%

Slope = N/A # Animals/Level = 20 Temperature = 20°C

Lab Springborn Life Sciences

48-Hour Dose Level pp/(X Mortality)  
130(0), 220(65), 360(100), 600(100), 1000(100)

Acc. 405399-05

Comments: \* BASED ON NOMINAL CONCENTRATIONS

M. WHITTEN  
8-2-89  
Supplemental

96-Hour LC<sub>50</sub>

LC<sub>50</sub> = PP ( 95% C.L. ) Con. Mort.(X) = \_\_\_\_\_  
Sol. Con. Mort.(X) = \_\_\_\_\_

Species \_\_\_\_\_

Slope = \_\_\_\_\_ # Animals/Level = \_\_\_\_\_ Temp. = \_\_\_\_\_

Lab \_\_\_\_\_

96-Hour Dose Level pp/(X Mortality)  
( ) , ( ) , ( ) , ( ) , ( ) , ( ) , ( ) , ( )

Acc. \_\_\_\_\_

Comments: \_\_\_\_\_

96-Hour LC<sub>50</sub>

LC<sub>50</sub> = PP ( 95% C.L. ) Con. Mort.(X) = \_\_\_\_\_  
Sol. Con. Mort.(X) = \_\_\_\_\_

Species \_\_\_\_\_

Slope = \_\_\_\_\_ # Animals/Level = \_\_\_\_\_ Temp. = \_\_\_\_\_

Lab \_\_\_\_\_

96-Hour Dose Level pp/(X Mortality)  
( ) , ( ) , ( ) , ( ) , ( ) , ( ) , ( ) , ( )

Acc. \_\_\_\_\_

Comments: \_\_\_\_\_